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Editorial

Jure Piškur (1960-2014)

I am shocked by the premature death of Jure Piškur, May 18th of this year, to cancer. In view of the little time available to obtain an obituary, I include in this issue a very personal letter written by Jure himself as he approached his impending fate. Like most who knew Jure, I admired his goodness, his genuine nature, his dedication to science and to education. Most importantly, in spite of his superb accomplishments, Jure remained a simple, generous, and inclusive person. He was a model for us all.

M.A. Lachance, Editor

Letter to the Editor

Some strains of many ascomycetous and basidiomycetous yeast species secrete (glyco)proteins (mycocins, killer toxins) having fungicidal or fungistatic action (Golubev, 2006). Mycocin sensitivity is restricted by organisms taxonomically related to mycocinogenic strains. From this viewpoint the communications about mycocin activity against organisms non-related phylogenetically to mycocin producer cast doubt. As an example, killer activity of *Schizosaccharomyces pombe* Lindner against *Candida glabrata* (Anderson) Meyer et Yarrow and *Saccharomyces cerevisiae* Meyen ex Hansen has been reported (Bonilia-Salinas et al., 1995), though budding yeasts belong to Saccharomycotina subphylum whereas fission yeast do to Archiascomycotina (Kuramae et al., 2006). When screening VKM strains of the genus *Schizosaccharomyces* (36 strains of three species) for mycocinogeny, two strains of *S. pombe* were found that showed the activity. Five strains of this species are sensitive and the rest have neutral phenotype. These two mycocinogenic strains are not active against any

budding yeasts belonging to 13 species of 12 genera but they act against *Protomyces macrosporus*, *Taphrina bergeniae*, *T. carnea* and *T. tosquinetii*. All strains of *Schizosaccharomyces* spp, examined are insensitive to *Kluyveromyces waltii* mycocin (Kono and Himeno, 1997).

References:

- 1 Golubev W.I., 2006. Antagonistic interactions among yeasts. In: Biodiversity and Ecophysiology of Yeasts (eds. C.A. Rosa, G. Peter). Springer-Verlag pp. 197-219.
- 2 Bonilia-Salinas M., Lappe, P., Ulloa, M., Garcia-Garibay, M. & Gomez Ruiz, L., 1995. Isolation and identification of killer yeasts from sugar cane molasses. Lett. Appl. Microbiol. 21: 115-116.
- 3 Kuramae E. E., Robert V., Snel B., Weiß M & Teun Boekhout T. Phylogenomics reveal a robust fungal tree of life. FEMS Yeast Res. 6: 1213-1220.
- 4 Kono I. & Himeno K., 1997. A novel killer yeast effective on *Schizosaccharomyces pombe*. Biosci. Biotech. Biochem. 61: 563-564.

Paper sent to press in 2014.

- 1 Kopecká M 2014 Microtubules and actin cytoskeleton of human potentially pathogenic yeast *Cryptococcus neoformans* as targets for antifungals. Chemotherapy (Karger, Switzerland).

We are grateful to Matti Korhola (Helsinki), Alexander Rapoport (Riga) and Joseph Schacherer (Strasbourg), Isabelle Masneuf-Pomarede (Bordeaux) for making it possible to visit their labs in November 2013 and February 2014.

The following are papers for 2014 or submitted.

- 1 Naumov GI, Naumova ES 2014 Polymeric lactose fermentation genes in the yeast *Kluyveromyces lactis*: a new locus *LAC3*. Doklady Biological Sciences 455: 106-108. © Pleiades Publishing, Ltd.
- 2 Naumova ES, Sadykova AZh, Martynenko NN, Naumov GI 2014 Molecular polymorphism of β -fructosidase *SUC* genes in the yeast *Saccharomyces*. Molecular Biology (Moscow), 48(4) (in press).

Molecular polymorphism of *SUC* genes encoding β -fructosidase has been investigated in the yeast genus *Saccharomyces*. We have determined nucleotide sequences of subtelomeric *SUC3*, *SUC5*, *SUC7*, *SUC8*, *SUC9*, *SUC10* genes of *S. cerevisiae* and *SUCa* gene of *S. arboricola*. Comparisons of nucleotide sequences of all known *SUC* genes revealed predominance of transitions C→T in the third codon position, which are silent. The aminoacids sequences of β -fructosidases studied have identity of 88–100%. Most divergent are *SUCa* (*S. arboricola*) and *SUCb*

(*S. bayanus*), having amino acid identity with the other *SUC* proteins less than 92%. It was determined that accumulation of the polymeric *SUC* genes takes place in industrial populations of *S. cerevisiae*, while the other *Saccharomyces* species (*S. arboricola*, *S. bayanus*, *S. cariocanus*, *S. kudriavzevii*, *S. mikatae* and *S. paradoxus*) each harbor only one *SUC* gene. Subtelomeric repeats of β -fructosidase *SUC* genes could appear in the genome of *S. cerevisiae* under the effect of selection in the course of their domestication.

- 3 Naumov GI, Kondratieva VI, Naumova ES 2014 Hybrid sterility of the yeast *Schizosaccharomyces pombe*: genetic genus and many species in statu nascendi? Microbiology (Moscow), 83 (6) (in press).
- 4 Naumov GI, Naumova ES, Glushakova AM, Kachalkin AV, Chernov Iyu 2014 Finding of dairy yeast *Kluyveromyces lactis* var. *lactis* in nature. Microbiology (Moscow) (submitted).

IV Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL, USA. Communicated by C. P. Kurtzman <cletus.kurtzman@ars.usda.gov>.

Recent publications.

- 1 Kurtzman CP & Robnett CJ 2013 Description of *Ambrosiozyma oregonensis* sp. nov., and reassignment of *Candida* species of the *Ambrosiozyma* clade to *Ambrosiozyma kashinagacola* f.a., comb. nov., *Ambrosiozyma llanquihuensis* f.a., comb. nov., *Ambrosiozyma maleeae* f.a., comb. nov., *Ambrosiozyma pseudovanderkluftii* f.a., comb. nov., and *Ambrosiozyma vanderkluftii* f.a., comb. nov. Int J Syst Evol Microbiol 63:3877–3883 - doi: 10.1099/ijms.0.055293-0.

Ambrosiozyma oregonensis sp. nov. is described from two strains, one isolated from a mountain stream in Oregon, USA (NRRL Y-6106T, CBS 5560T), and a second (NRRL YB-4169) from an unknown substrate from Marion, Illinois, USA. The species forms four hat-shaped ascospores in each deliquescent ascus and appears to be homothallic. Abundant true hyphae are produced with some having apparent dolipore-like septa. Analyses of nuclear gene sequences for the D1/D2 domains of large-subunit rRNA, small-subunit rRNA, translation elongation factor-1 α , and subunits

B1 and B2 of RNA polymerase II show the proposed novel species to be distinct from other species of the *Ambrosiozyma* clade. Because of their placement in the *Ambrosiozyma* clade, *Candida kashinagacola*, *Candida llanquihuensis*, *Candida maleeae*, *Candida pseudovanderkluftii* and *Candida vanderkluftii* are reassigned to the genus *Ambrosiozyma* as new combinations, and the description of the genus *Ambrosiozyma* is emended to reflect the resulting changes in phenotypic characters.

- 2 Maragos CM, Kurtzman C, Busman M, Price N & McCormick S 2013 Development and evaluation of monoclonal antibodies for the glucoside of T-2 toxin (t2-glc). Toxins (Basel) 19:1299-313 - doi: 10.3390/toxins5071299.

The interactions between fungi and plants can yield metabolites that are toxic in animal systems. Certain fungi are known to produce sesquiterpenoid

trichothecenes, such as T-2 toxin, that are biotransformed by several mechanisms including glucosylation. The glucosylated forms have been

found in grain and are of interest as potential reservoirs of T-2 toxin that are not detected by many analytical methods. Hence the glucosides of trichothecenes are often termed "masked" mycotoxins. The glucoside of T-2 toxin (T2-Glc) was linked to keyhole limpet hemocyanin and used to produce antibodies in mice. Ten monoclonal antibody (Mab)-producing hybridoma cell lines were developed. The Mabs were used in immunoassays to detect T2-Glc

and T-2 toxin, with midpoints of inhibition curves (IC50s) in the low ng/mL range. Most of the Mabs demonstrated good cross-reactivity to T-2 toxin, with lower recognition of HT-2 toxin. One of the clones (2-13) was further characterized with in-depth cross-reactivity and solvent tolerance studies. Results suggest Mab 2-13 will be useful for the simultaneous detection of T-2 toxin and T2-Glc.

- 3 Hughes SR, Bang SS, Cox EJ, Schoepke A, Ochwat K, Pinkelman R, Nelson D, Qureshi N, Gibbons WR, Kurtzman CP, Bischoff KM, Liu S, Cote GL, Rich JO, Jones MA, Cedeño D, Doran-Peterson J, Riaño-Herrera NM, Rodríguez-Valencia N & López-Núñez JC 2013 Automated UV-C mutagenesis of *Kluyveromyces marxianus* NRRL Y-1109 and selection for microaerophilic growth and ethanol production at elevated temperature on biomass sugars. J Lab Autom. 18:276-290 - doi: 10.1177/2211068213480037.

The yeast *Kluyveromyces marxianus* is a potential microbial catalyst for fuel ethanol production from a wide range of biomass substrates. To improve its growth and ethanol yield at elevated temperature under microaerophilic conditions, *K. marxianus* NRRL Y-1109 was irradiated with UV-C using automated protocols on a robotic platform for picking and spreading irradiated cultures and for processing the resulting plates. The plates were incubated under anaerobic conditions on xylose or glucose for 5 mo at 46 °C. Two *K. marxianus* mutant strains (designated 7-1 and 8-1) survived and were isolated from the glucose plates. Both mutant strains, but not wild type, grew aerobically on glucose at 47 °C. All strains grew anaerobically at 46 °C on glucose, galactose,

galacturonic acid, and pectin; however, only 7-1 grew anaerobically on xylose at 46 °C. *Saccharomyces cerevisiae* NRRL Y-2403 did not grow at 46 °C on any of these substrates. With glucose as a carbon source, ethanol yield after 3 d at 46 °C was higher for 8-1 than for wild type (0.51 and 0.43 g ethanol/g glucose, respectively). With galacturonic acid as a carbon source, the ethanol yield after 7 d at 46 °C was higher for 7-1 than for wild type (0.48 and 0.34 g ethanol/g galacturonic acid, respectively). These mutant strains have potential application in fuel ethanol production at elevated temperature from sugar constituents of starch, sucrose, pectin, and cellulosic biomass.

- 4 Kurtzman CP 2014 Use of gene sequence analyses and genome comparisons for yeast systematic. Int J Syst Evol Microbiol 64:325–332 - doi: 10.1099/ijms.0.054197-0.

Detection, identification and classification of yeasts have undergone a major transformation in the past decade and a half following application of gene sequence analyses and genome comparisons. Development of a database (barcode) of easily determined gene sequences from domains 1 and 2 (D1/D2) of large subunit rRNA and from the internal transcribed spacer (ITS) now permits many laboratories to identify species accurately and this has led to a doubling in the number of known species of yeasts over the past decade. Phylogenetic analysis of

gene sequences has resulted in major revision of yeast systematics, resulting in redefinition of nearly all genera. Future work calls for application of genomics to refine our understanding of the species concept and to provide a better understanding of the boundaries of genera and higher levels of classification. This increased understanding of phylogeny is expected to allow prediction of the genetic potential of various clades and species for biotechnological applications and adaptation to environmental changes.

- 5 Janisiewicz WJ, Jurick WM, Peter KA, Kurtzman CP & Buyer JS 2014 Yeasts associated with plums and their potential for controlling brown rot after harvest. Yeast - doi: 10.1002/yea.3009.

Bacterial and yeast antagonists isolated from fruit surfaces have been effective in controlling various post-harvest diseases, and several microbial

antagonists have been developed into commercial products. Our knowledge of the fruit microbial community, with the exception of grapes, apples and

some citrus fruit, is rudimentary and the potential of the resident yeasts for biocontrol remains largely unknown. We determined the occurrence of yeasts on plum surfaces during fruit development from the pre-hardening stage until harvest for 2 years. A total of 16 species from 13 genera were isolated. Species from three genera, basidiomycetes *Rhodotorula* (29.5%) and *Sporidiobolus* (24.7%) and the dimorphic ascomycete genus *Aureobasidium* (24.7%), constituted 78.7% of all isolations and were recovered throughout fruit development, while *Cryptococcus* spp. constituted only 6.2% of the total plum isolates. The yeast community in the final sampling was significantly different from the first three samplings, reflecting a rapidly changing fruit habitat during the maturation of fruit. For example, *Hanseniaspora*, *Pichia*,

Zygosaccharomyces and *Wickerhamomyces* occurred only on the most mature fruit. Screening of the yeasts for antagonistic activity against *Monilinia fructicola*, a fungus that causes brown rot, revealed a range of biocontrol activities. Several isolates provided complete control of the decay on plums, challenged with a pathogen suspension of 10^3 conidia/ml and > 90% of control on fruit inoculated with the pathogen at a concentration 10 times higher. Some of the best antagonists included *A. pullulans* and *R. phylloplana*. Populations of both of these antagonists increased rapidly by several orders of magnitude in wounds of plums incubated at 24°C and 4°C. Our results indicate that plum surfaces harbour several yeast species, with excellent potential for use in biological control of brown rot of stone fruits.

- 6 Kurtzman CP & Robnett CJ 2014 Three new anascosporic genera of the Saccharomycotina: *Danielozyma* gen. nov., *Deakozyma* gen. nov. and *Middelhovenomyces* gen. nov. Antonie van Leeuwenhoek 105:933-942 - doi: 10.1007/s10482-014-0149-9.

Three new non-ascosporic, ascomycetous yeast genera are proposed based on their isolation from currently described species and genera. Phylogenetic placement of the genera was determined from analysis of nuclear gene sequences for D1/D2 large subunit rRNA, small subunit rRNA, translation elongation factor-1 α and RNA polymerase II, subunits B1 and B2. The new taxa are: *Deakozyma* gen. nov., type species *Deakozyma indianensis* sp. nov. (type strain

NRRL YB-1937, CBS 12903); *Danielozyma* gen. nov., type species *Danielozyma ontarioensis* comb. nov. (type strain NRRL YB-1246, CBS 8502); *D. litseae* comb. nov. (type strain NRRL YB-3246, CBS 8799); *Middelhovenomyces* gen. nov., type species *Middelhovenomyces tepae* comb. nov. (type strain NRRL Y-17670, CBS 5115) and *M. petrohuensis* comb. nov. (type strain NRRL Y-17663, CBS 8173).

- 7 Daniel HM, Lachance MA & Kurtzman CP 2014 On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription. Antonie van Leeuwenhoek. 2014 Apr 19 [Epub ahead of print]

Multigene phylogenies have been instrumental in revising the classification of ascosporic (teleomorph) yeasts in a natural system based on lines of descent. Although many taxonomic changes have already been implemented for teleomorph taxa, this is not yet the case for the large genus *Candida* and smaller anascosporic (anamorph) genera. In view of the recently introduced requirement that a fungal species or higher taxon be assigned only a single valid name under the new International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), the current species of *Candida* and other anamorph yeast genera must undergo revision to make genus membership consistent with phylogenetic affinities. A review of

existing data and analyses shows that certain *Candida* species may be assigned to teleomorph genera with high confidence using multigene phylogenies. *Candida* species that form well-circumscribed phylogenetic clades without any teleomorph member justify the creation of new genera. However, a considerable number of *Candida* species sit at the end of isolated and often long branches, and hence cannot be assigned to larger species groups. They should be maintained in *Candida* sensu lato until studied by multigene analyses in datasets with comprehensive taxon sampling. The principle of name stability has to be honoured to the largest extent compatible with a natural classification of *Candida* species.

Paper to be presented at the The American Society of Brewing Chemists (ASBC) meeting in Chicago in June 2014.

1 Nayyar A, Walker GM, Canetta E, Wardrop F and Adya AK Correlation of Cell Surface Properties of Industrial Yeast Strains to their Functional Role in Fermentations

Adhesion properties are known to play important roles in governing many essential aspects of the life cycles of microorganisms like sexual reproduction, cellular aggregation during processes such as flocculation and bio-film formation, invasion and/or pathogenic behaviour, and many others. Adhesion properties, by far, are dependent on the characteristics of the cellular surface, usually the outer layer of the cell wall. Microorganisms can adjust their adhesion properties by changing the structure of their external cell surface. Flocculence, is the ability of the yeast cells to flocculate under optimal conditions, which is a cell wall property independent of its environment. Thus, when we study flocculation we need to consider the cell wall properties. The flocculation behaviour of four industrial *Saccharomyces cerevisiae* strains expressing either *Flo1* or *NewFlo* phenotype were examined. These were strains employed for brewing, champagne production, winemaking and fuel alcohol production. The behaviour of brewing and champagne strains differed in terms of their cell-surface hydrophobicity, cell-surface charge, and presence of adhesins and cell-wall binding sites (mannose residues) which likely impinge on their flocculation behaviour. The brewing yeast strain exhibited the highest degree of flocculation amongst all the strains, and it was accompanied with a concomitantly high hydrophobicity index of 66%. This supports our hypothesis that cell surface hydrophobicity plays a major role in controlling yeast flocculation behaviour in the fermenter. Equally important is cell-surface

charge which were shown in highly flocculent brewing strains to possess very high negative charge. From the studies, it was observed that high cell surface hydrophobicity, bonds between the adhesins and mannose residues (stabilised by Ca^{2+} ions) and finally the surface topography of yeast strains are responsible for maintaining flocs during the fermentation process. We have additionally observed that in contrast to wine and fuel alcohol yeast strains, brewing and champagne strains exhibit increased cell-wall mannose concentrations from the early stationary phase to the late stationary phase. This correlates with simultaneous increase in flocculation ability. Brewing yeasts may therefore be characterised by a high density of mannose residues on their outer cell-walls. In addition, we found that the brewing yeast strain studied had a high lectin density (3.65×10^6 lectins/cell) compared with the champagne strain (2.44×10^6 lectins/cell). Yeast adhesion properties and cell wall physiology were further investigated at the nanoscale using Atomic Force Microscopy (AFM). For example, surface roughness, Young's Modulus, and adhesion energy of industrial yeast strains determined by AFM provided new information regarding yeast cell walls and physiological behaviour. The work will further aid in greater understanding about the onset of yeast flocculation, and the vital role that cell surface hydrophobicity, cell surface charge, surface topography together with the density of adhesins on the yeast cell surface play for the brewing processes in fermentation.

Recent publications.

1 Schnierda T, FF Bauer, B Divol, E van Rensburg and JF Görgens 2014 Optimization of carbon and nitrogen medium components for biomass production using non-*Saccharomyces* wine yeasts. Lett Appl Microbiol 58:478-85.

The impact of different nitrogen and carbon sources on biomass production of the non-*Saccharomyces* wine yeast species *Lachancea thermotolerans*, *Metschnikowia pulcherrima* and *Issatchenkia orientalis* was assessed. Using a

molasses-based medium, yeast extract and corn steep liquor as well as ammonium sulphate and diammonium phosphate (DAP) as nitrogen sources were compared in shake-flask cultures. A medium with 20 g l⁻¹ sugar (diluted molasses) and 500 mg l⁻¹ total yeast

assimilable nitrogen, from yeast extract, gave the highest biomass concentrations and yields. Invertase pretreatment was required for cultures of *M. pulcherrima* and *I. orientalis*, and respective biomass yields of 0.7 and 0.8 g g⁻¹ were achieved in aerobic bioreactor cultures. The absence of ethanol production suggested Crabtree-negative behaviour by these yeasts, whereas Crabtree-positive behaviour by *L. thermotolerans* resulted in ethanol and biomass concentrations of 5.5 and 11.1 g l⁻¹, respectively.

- 2 Franken J, Brandt BA, Tai SL, Bauer FF 2014 Biosynthesis of levan, a bacterial extracellular polysaccharide, in the yeast *Saccharomyces cerevisiae*. PLoS One 8(10):e77499.

Levans are fructose polymers synthesized by a broad range of micro-organisms and a limited number of plant species as non-structural storage carbohydrates. In microbes, these polymers contribute to the formation of the extracellular polysaccharide (EPS) matrix and play a role in microbial biofilm formation. Levans belong to a larger group of commercially important polymers, referred to as fructans, which are used as a source of prebiotic fibre. For levan, specifically, this market remains untapped, since no viable production strategy has been established. Synthesis of levan is catalysed by a group of enzymes, referred to as levansucrases, using sucrose as substrate. Heterologous expression of levansucrases has been notoriously difficult to achieve in *Saccharomyces cerevisiae*. As a strategy, this study used an invertase (*Δsuc2*) null mutant and two separate, engineered, sucrose accumulating yeast strains as hosts for the expression of the levansucrase MIFT, previously cloned from *Leuconostoc*

SIGNIFICANCE AND IMPACT OF THE STUDY: Recent studies demonstrate that non-*Saccharomyces* yeasts confer positive attributes to the final composition of wine. However, optimal process conditions for their biomass production have not been described, thereby limiting commercial application. In this study, industrial media and methods of yeast cultivation were investigated to develop protocols for biomass production of non-*Saccharomyces* yeast starter cultures for the wine industry.

mesenteroides. Intracellular sucrose accumulation was achieved either by expression of a sucrose synthase (Susy) from potato or the spinach sucrose transporter (SUT). The data indicate that in both *Δsuc2* and the sucrose accumulating strains, the MIFT was able to catalyse fructose polymerisation. In the absence of the predicted MIFT secretion signal, intracellular levan accumulation was significantly enhanced for both sucrose accumulation strains, when grown on minimal media. Interestingly, co-expression of MIFT and SUT resulted in hyper-production and extracellular build-up of levan when grown in rich medium containing sucrose. This study presents the first report of levan production in *S. cerevisiae* and opens potential avenues for the production of levan using this well established industrial microbe. Furthermore, the work provides interesting perspectives when considering the heterologous expression of sugar polymerizing enzymes in yeast.

- 3 Rossouw D, Heyns EH, Setati ME, Bosch S, Bauer FF 2013 Adjustment of trehalose metabolism in wine *Saccharomyces cerevisiae* strains to modify ethanol yields. Applied and Environmental Microbiology 79:5197-207.

The ability of *Saccharomyces cerevisiae* to efficiently produce high levels of ethanol through glycolysis has been the focus of much scientific and industrial activity. Despite the accumulated knowledge regarding glycolysis, the modification of flux through this pathway to modify ethanol yields has proved difficult. Here, we report on the systematic screening of 66 strains with deletion mutations of genes encoding enzymes involved in central carbohydrate metabolism for altered ethanol yields. Five of these strains showing the most prominent changes in carbon flux were selected for further investigation. The genes were representative of trehalose biosynthesis (*TPS1*, encoding trehalose-6-phosphate synthase), central glycolysis (*TDH3*, encoding glyceraldehyde-3-phosphate dehydrogenase), the oxidative pentose phosphate pathway (*ZWF1*,

encoding glucose-6-phosphate dehydrogenase), and the tricarboxylic acid (TCA) cycle (*ACO1* and *ACO2*, encoding aconitase isoforms 1 and 2). Two strains exhibited lower ethanol yields than the wild type (*tps1Δ* and *tdh3Δ*), while the remaining three showed higher ethanol yields. To validate these findings in an industrial yeast strain, the *TPS1* gene was selected as a good candidate for genetic modification to alter flux to ethanol during alcoholic fermentation in wine. Using low-strength promoters active at different stages of fermentation, the expression of the *TPS1* gene was slightly upregulated, resulting in a decrease in ethanol production and an increase in trehalose biosynthesis during fermentation. Thus, the mutant screening approach was successful in terms of identifying target genes for genetic modification in commercial yeast strains with the aim of producing lower-ethanol wines.

- 4 Mostert TT, Divol B 2014 Investigating the proteins released by yeasts in synthetic wine fermentations. *Int J Food Microbiol* 171:108-18.

Proteins from various biological sources previously identified in wine play important roles in the functioning and survival of their producers and may exhibit oenological properties. Yeasts contribute significantly to the protein pool during and after alcoholic fermentation. While the extracellular proteins of *Saccharomyces cerevisiae*, the main wine yeast species, have been characterised, those of non-*Saccharomyces* yeasts remain restricted to a few enzymes. A more comprehensive insight into all proteins released during fermentation could improve our understanding of how yeasts survive and interact in mixed culture fermentations. This study aimed to characterise the exo-proteome of *Saccharomyces* and selected non-*Saccharomyces* yeasts in pure and mixed

cultures in a wine-like medium. While *S. cerevisiae* completed the fermentation rapidly, *Metschnikowia pulcherrima* hardly fermented and *Lachancea thermotolerans* fermented slowly but steadily. In sequential fermentations, the kinetics resembled those of the non-*Saccharomyces* yeasts for a period before switching to that of *S. cerevisiae*. Identification of the proteins present in wine at the end of fermentation using mass fingerprinting revealed the large diversity of proteins secreted and the influence of yeast interactions therein. The fermentation kinetics observed could partially be explained by the extent of the contribution of the different yeast to the protein content.

- 5 Viktor MJ, Rose SH, van Zyl WH, Viljoen-Bloom M 2013 Raw starch conversion by *Saccharomyces cerevisiae* expressing *Aspergillus tubingensis* amylases. *Biotechnol Biofuels* 6(1):167.

BACKGROUND: Starch is one of the most abundant organic polysaccharides available for the production of bio-ethanol as an alternative transport fuel. Cost-effective utilisation of starch requires consolidated bioprocessing (CBP) where a single microorganism can produce the enzymes required for hydrolysis of starch, and also convert the glucose monomers to ethanol. **RESULTS:** The *Aspergillus tubingensis* T8.4 α -amylase (*amyA*) and glucoamylase (*glaA*) genes were cloned and expressed in the laboratory strain *Saccharomyces cerevisiae* Y294 and the semi-industrial strain, *S. cerevisiae* Mnu α 1. The recombinant AmyA and GlaA displayed protein sizes of 110-150 kDa and 90 kDa, respectively, suggesting significant glycosylation in *S. cerevisiae*. The Mnu α 1[AmyA-GlaA] and Y294[AmyA-GlaA] strains were able to utilise 20 g l⁻¹ raw corn starch as sole carbohydrate source, with ethanol titers of 9.03 and

6.67 g l⁻¹ (0.038 and 0.028 g l⁻¹ h⁻¹), respectively, after 10 days. With a substrate load of 200 g l⁻¹ raw corn starch, Mnu α 1[AmyA-GlaA] yielded 70.07 g l⁻¹ ethanol (0.58 g l⁻¹ h⁻¹) after 120 h of fermentation, whereas Y294[AmyA-GlaA] was less efficient at 43.33 g l⁻¹ ethanol (0.36 g l⁻¹ h⁻¹). **CONCLUSIONS:** In a semi-industrial amyolytic *S. cerevisiae* strain expressing the *A. tubingensis* α -amylase and glucoamylase genes, 200 g l⁻¹ raw starch was completely hydrolysed (saccharified) in 120 hours with 74% converted to released sugars plus fermentation products and the remainder presumably to biomass. The single-step conversion of raw starch represents significant progress towards the realisation of CBP without the need for any heat pretreatment. Furthermore, the amylases were produced and secreted by the host strain, thus circumventing the need for exogenous amylases.

VII Laboratorio de Microbiología Aplicada y Biotecnología, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Universidad Nacional del Comahue - CONICET, (8400) Quintral 1250, Bariloche, Rio Negro, Argentina. Communicated by Diego Libkind Frati <diego.libkind@gmail.com>.

Recent publications.

- 1 Tognetti C, Moliné M, van Broock, M, Libkind D 2013 Favored isolation and rapid identification of *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) from environmental samples. *J Basic Microbiol* 53:1-7.
- 2 David-Palma, M, Libkind, D, Sampaio, JP 2014 Global distribution, diversity hotspots and niche transitions of an astaxanthin-producing eukaryotic microbe. *Molec Ecol* 23:921-932.

- 3 Peris D, Sylvester K, Libkind D, Gonçalves P, Sampaio JP Alexander W, Hittinger C 2014 Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. *Molec Ecol* 23:2031-2045.

Book chapters:

- 4 de García V, Moliné M, Libkind D, Giraudo MR 2013 Cold adapted yeasts in Patagonian habitats. In: *Cold-adapted yeasts: Biodiversity, adaptation strategies and biotechnological significance*. Editors: Pietro Buzzini and Rosa Margesin Publisher: Springer Verlag, Berlin Heidelberg. Chapter 6. ISBN 978-3-642-39680-9. pp. 123-148.
- 5 Moliné M, Libkind D, de García V, Giraudo MR 2013 Production of pigments and photo-protective compounds by cold-adapted yeasts. In: *Cold-adapted yeasts: biodiversity, adaptation strategies and biotechnological significance*. Editors: Pietro Buzzini and Rosa Margesin Publisher: Springer Verlag, Berlin Heidelberg. Chapter 9. ISBN 978-3-642-39680-9. pp. 193-224.
- 6 Jones G, Southworth EB, Libkind D, Marvanová L 2014 Freshwater Basidiomycota. In: *Freshwater fungi and fungus-like organisms*, Editor: Jones G, Pang K-L, Hyde K. De Gruyter Series: Marine and Freshwater Botany. Ch. 4. De Gruyter Editorial. In press.
- 7 Libkind D, Russo G, van Broock MR 2014 Yeasts from extreme aquatic environments: hyperacidic freshwaters. In: *Freshwater fungi and fungus-like organisms*, Editor: Jones G, Pang K-L, Hyde K. De Gruyter Series: Marine and Freshwater Botany. Ch. 19. De Gruyter Editorial. In press.

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The following papers were recently published or accepted for publication.

- 1 Leducq J-B, Guillaume C, Samani P, Dubé AK, Sylvester K, James B, Almeida P, Sampaio JP, Hittinger CT, Bell G, Landry CR 2014 Local climatic adaptation in a widespread microorganism. *Proc Royal Soc B* 281:20132472.

Exploring the ability of organisms to locally adapt is critical for determining the outcome of rapid climate changes, yet few studies have addressed this question in microorganisms. We investigated the role of a heterogeneous climate on adaptation of North American populations of the wild yeast *Saccharomyces paradoxus*. We found abundant among-strain variation for fitness components across a range of temperatures, but this variation was only partially explained by climatic variation in the distribution area. Most of fitness variation was

explained by the divergence of genetically distinct groups, distributed along a north-south cline, suggesting that these groups have adapted to distinct climatic conditions. Within-group fitness components were correlated with climatic conditions, illustrating that even ubiquitous microorganisms locally adapt and harbour standing genetic variation for climate-related traits. Our results suggest that global climatic changes could lead to adaptation to new conditions within groups, or changes in their geographical distributions.

- 2 David-Palma M, Libkind D, Sampaio JP 2014 Global distribution, diversity hotspots and niche transitions of an astaxanthin-producing eukaryotic microbe. *Mol Ecol* 23:921-932.

Microbes establish very diverse but still poorly understood associations with other microscopic or macroscopic organisms that do not follow the more conventional modes of competition or mutualism. *Phaffia rhodozyma*, an orange-coloured yeast that produces the biotechnologically relevant carotenoid astaxanthin, exhibits a Holarctic association with birch

trees in temperate forests that contrasts with the more recent finding of a South American population associated with *Nothofagus* (southern beech) and with stromata of its biotrophic fungal parasite *Cyttaria* spp. We investigated whether the association of *Phaffia* with *Nothofagus*-*Cyttaria* could be expanded to Australasia, the other region of the world where

Nothofagus are endemic, studied the genetic structure of populations representing the known worldwide distribution of *Phaffia* and analysed the evolution of the association with tree hosts. The phylogenetic analysis revealed that *Phaffia* diversity in Australasia is much higher than in other regions of the globe and that two endemic and markedly divergent lineages seem to represent new species. The observed genetic diversity correlates with host tree genera rather than with geography, which suggests that adaptation to the

different niches is driving population structure in this yeast. The high genetic diversity and endemism in Australasia indicate that the genus evolved in this region and that the association with *Nothofagus* is the ancestral tree association. Estimates of the divergence times of *Phaffia* lineages point to splits that are much more recent than the break-up of Gondwana, supporting that long-distance dispersal rather than vicariance is responsible for observed distribution of *P. rhodozyma*.

- 3 Peris D, Sylvester K, Libkind D, Gonçalves P, Sampaio JP, Alexander WG, Hittinger CT 2014 Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. *Molecular Ecology* 23:2031–2045.

Reticulate evolution can be a major driver of diversification into new niches, especially in disturbed habitats and at the edges of ranges. Industrial fermentation strains of yeast provide a window into these processes, but progress has been hampered by a limited understanding of the natural diversity and distribution of *Saccharomyces* species and populations. For example, lager beer is brewed with *Saccharomyces pastorianus*, an allopolyploid hybrid of *S. cerevisiae* and *S. eubayanus*, a species only recently discovered in Patagonia, Argentina. Here, we report that genetically diverse strains of *S. eubayanus* are readily isolated from Patagonia, demonstrating that the species is well established there. Analyses of multilocus sequence data strongly suggest that there are two diverse and highly differentiated Patagonian populations. The low nucleotide diversity found in the *S. eubayanus* moiety of hybrid European brewing

strains suggests that their alleles were drawn from a small subpopulation that is closely related to one of the Patagonian populations. For the first time, we also report the rare isolation of *S. eubayanus* outside Patagonia, in Wisconsin, USA. In contrast to the clear population differentiation in Patagonia, the North American strains represent a recent and possibly transient admixture of the two Patagonian populations. These complex and varied reticulation events are not adequately captured by conventional phylogenetic methods and required analyses of Bayesian concordance factors and phylogenetic networks to accurately summarize and interpret. These findings show how genetically diverse eukaryotic microbes can produce rare but economically important hybrids with low genetic diversity when they migrate from their natural ecological context.

- 4 Almeida P, Gonçalves C, Teixeira S, Libkind D, Bontrager M, Masneuf-Pomarède I, Albertin W, Durrens P, Sherman D, Marullo P, Hittinger CT, Gonçalves P, Sampaio JP 2014 A Gondwanan imprint on global diversity and domestication of wine and cider yeast *Saccharomyces uvarum*. *Nature Communications* (in press).

In addition to *Saccharomyces cerevisiae*, the cryotolerant yeast species *S. uvarum* is also used for wine and cider fermentation but nothing is known about its natural history. Here we use a population genomics approach to investigate its global phylogeography and domestication fingerprints using a collection of isolates obtained from fermented beverages and from natural environments on five continents. South American isolates contain more genetic diversity than that found in the Northern Hemisphere. Moreover, coalescence analyses suggest

that a Patagonian sub-population gave rise to the Holarctic population through a recent bottleneck. Holarctic strains display multiple introgressions from other *Saccharomyces* species, those from *S. eubayanus* being prevalent in European strains associated with human-driven fermentations. These introgressions are absent in the large majority of wild strains and gene ontology analyses indicate that several gene categories relevant for wine fermentation are overrepresented. Such findings constitute a first indication of domestication in *S. uvarum*.

Letter to the Editor

An update on substrate specificities of α -glucosidases in *Saccharomyces cerevisiae*

There are two major α -glucosidases in the yeast *S. cerevisiae*, namely maltase (EC3.2.1.20), responsible for the hydrolysis α -(1,4) glucosidic bond of the disaccharide maltose, and isomaltase (EC3.2.1.10) responsible for the hydrolysis of α -(1,6) disaccharide of isomaltose. (Yamamoto et al 2004, Khan and Eaton 1967).

A recent paper by Deng Xu et al (2014) describes in detail the properties of isomaltases encoded by the IMA genes isolated and characterized by Teste et. al. (2010). These authors have tested in vitro the substrate specificities of four isomaltases, and part of the results can be described as follows: (1) All four Ima proteins preferentially hydrolyse α -1,6 disaccharides such as isomaltose; (2) all Ima proteins were totally inactive on maltose; (3) Maltose is not a substrate of yeast isomaltase, but it acts as an inhibitor of this enzyme; and (4) all Ima proteins show activity on sucrose.

Therefore, it is clear from the work of Xu Deng et. al. and the previous work that maltases are specific for the hydrolysis of maltose and isomaltases are specific for the hydrolysis of isomaltose and α -methylglucoside. And both of these α -glucosidases

show activity on sucrose.

References:

- 1 Deng Xu, Petitjean M, Teste MA, Kooli W, Tranier S, Francois JM and Parrou JL 2014 Similarities and differences in the biochemical and enzymological properties of the four isomaltases from *Saccharomyces cerevisiae*, Febs Open Bio 4:200-212
- 2 Teste MA, Francois JM & Parrou JL 2010 characterization of a new multigene family encoding isomaltases in the yeast *Saccharomyces cerevisiae*, the IMA family. J Biol Chem 285:26815-26824
- 3 Yamamoto K, Nakayama A, Yamamoto Y & Tabata S. 2004 Val216 decides the substrate specificity of α -glucosidase in *Saccharomyces cerevisiae*. Eur J Biochem 271:3414-4320.
- 4 Khan NA & Eaton NR . 1967 Purification and characterization of maltase and α -methylglucosidase from yeast. Biochim Biophys Acta 146:173-178.

Recent publication.

- 1 Calvey CH, Willis LB & Jeffries TW 2014 An optimized transformation protocol for *Lipomyces starkeyi*. Curr Genet 1–8 - doi:10.1007/s00294-014-0427-0

We report the development of an efficient genetic transformation system for *Lipomyces starkeyi* based on a modified lithium acetate transformation protocol. *L. starkeyi* is a highly lipogenic yeast that grows on a wide range of substrates. The initial transformation rate for this species was extremely low, and required very high concentrations of DNA. A systematic approach for optimizing the protocol resulted in an increase in the transformation efficiency by four orders of magnitude. Important parameters included cell

density, the duration of incubation and recovery periods, the heat shock temperature, and the concentration of lithium acetate and carrier DNA within the transformation mixture. We have achieved efficiencies in excess of 8,000 transformants/ μ g DNA, which now make it possible to screen libraries in the metabolic engineering of this yeast. Metabolic engineering based on this transformation system could improve lipogenesis and enable formation of higher value products.

Forthcoming book chapter.

- 1 Enrique Javier Carvajal Barriga, Patricia Portero Barahona, Carolina Tufiño, Bernardo Bastidas, Cristina Guamán-Burneo, Larissa Freitas & Carlos Rosa. An overview of the yeast biodiversity in the Galápagos Islands and other Ecuadorian regions.

One of the most emblematic natural regions for studies of evolution and biodiversity in the world is the Galápagos Islands, which is the inspiring environment where the naturalist Charles Darwin was moved to propose what eventually became the Theory of the Origin of Species launched in the 19th Century. This Archipelago has been formed by subaquatic volcanic activity around 5 million years ago. The plant and animal populations settled on this group of 21 islands and 107 rocks and islets were introduced mainly by the sea currents and winds that reached the emerging lands in this equatorial region of the sea. The study of plants and endemic species of animals has fascinated biologists for decades. Giant turtles, finches, marine and terrestrial iguanas and boobies have been the center of studies, as well as other birds and flora of the region. Many adaptations and evolution evidences were found in the macrobiota adapted to the particular environments of each island in the archipelago. However, not much attention was paid to the microorganisms and, in particular, to yeast biodiversity in the islands. In 2009 in an effort to address this scientific shortfall, a prospective study was started by an Ecuadorian-Brazilian-Spanish team that visited four human-inhabited islands (i.e. Floreana, San Cristóbal, Santa Cruz and Isabela). The substrates chosen by the researchers were mainly flowers from *Datura* and *Ipomoea* genera, as well as *Opuntia* fruits and leaves. Moreover, unique substrates like endemic tree's exudates or even giant turtle's and marine iguana's feces were also taken.

Flowers, insect, fungus and rotten vegetal matter were also part of the substrates chosen by the expeditionaries. The resulting prospection yielded more than 800 yeast isolates. Most of those yeasts have been identified by sequencing of the LSU or the 26S rDNA gene. Among the yeasts recovered, there are several novel yeast species such as *Saccharomycopsis fodiens* and *Kodamaea transpacificae*, and other hitherto non-described ones. About 31% of the yeast biota in the islands is coincident with the species found in Ecuador mainland. Most of the yeast species are hitherto not found in the mainland since 2006 when the Catholic University Yeasts Collection (CLQCA) initiated its identification, characterization and preservation activities, devoted to yeast. Currently this yeast collection represents the most complete deposit of wild species from Ecuador. A comparison between the yeast biodiversity in the islands with the yeasts biodiversity in Ecuadorian mainland is done in this chapter in order to draw a first line of understanding of the adaptability, biogeography and interaction of species in an insular territory located about 1000 Km from the nearest South American mainland coasts. Moreover, an overview of the yeast biodiversity of mainland Ecuador's ecosystems is addressed in this chapter in order to establish the comparisons and the extent in which the closest mainland has had influence in current microbial (yeast) biodiversity in this relatively recently formed archipelago in the Pacific Ocean.

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Recent publications.

- 1 Streltzkiy RA, Glushakova AM, Zavgorodnyaya YuA, Demin VV, Chernov IYu 2013 Detection of 3-indoleacetic acid in culture fluid of yeasts from different ecological groups. Mikologiya i fitopatologiya (Mycology and Phytopathology) 47:116–119 (in Russian).

Fourteen yeast strains belonging to 8 species isolated from phyllosphere, roots, soil and flowers were investigated. 3-Indoleacetic acid (IAA) was detected by chromatographic analysis in culture fluid of all strains. Its concentration was essentially different in various species and correlated with their

ecological features. The maximal concentration of IAA was found at epiphytic (*Rhodotorula mucilaginosa*, *Cryptococcus wieringae*), minimal – at euribiotic (*Wickerhamomyces anomalus*) and sugarbiontic (*Saccharomyces paradoxus*) species.

- 2 Chernov IYu, Glushakova AM, Kachalkin AV 2013 Annotated list of yeasts from Moscow region. *Mikologiya i fitopatologiya (Mycology and Phytopathology)* 47:103–115 (in Russian).
Annotated list of yeasts species found in different natural substrates in Moscow Region during several decades of monitoring investigations is presented. This is the first attempt to compile considerably complete list of yeast species for hole geographical region. The most of species was identified by sequencing of D1/D2 domain of 26S rDNA. The peculiarities of taxonomic composition of yeasts in Moscow Region are discussed.
- 3 Glushakova AM, Zhyoltikova TM, Chernov IYu 2013 Yeasts associated with pollen of anemogamous plants. *Mikologiya i fitopatologiya (Mycology and Phytopathology)* 47:294–299 (in Russian).
Yeasts number and diversity on pollen of some anemogamous plants being leading pollen allergens in a midland of Russia were studied: birch (*Betula pendula*), alder (*Alnus glutinosa*), avellan (*Corylus avellana*) and oak (*Quercus robur*). Experiments were carried out in city, forest-park and forest zones. In 5 years of monitoring 16 yeast species were isolated from pollen of anemogamous trees. Throughout the entire period of studying *Candida oleophila*, *Cryptococcus magnus*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa* dominated on pollen. It was shown that yeasts communities associated with pollen of anemogamous trees in various habitats were formed of epiphytic and euribiotic ascomycetous and basidiomycetous species. The average yeast population on pollen was found to be 10^3 CFU/g.
- 4 Glushakova AM, Kachalkin AV, Chernov IYu 2014 Yeasts in the flowers of entomophilic plants of the Moscow region. *Microbiology* 83:125–134.
Dynamics of abundance and diversity of epiphytic yeasts in entomophilic flowers of 28 species of meadow, forest, and cultivated plants throughout their blooming period was determined. The number of yeasts in the flowers was shown to increase gradually during the vegetation period, and reached the maximum during summer–autumn. The total abundance and ratio of the yeast species in the flowers depended entirely on the blooming time, rather than on the taxonomic position of the plants. Three stages of development of the entomophilic yeast complexes during the vegetation period may be discerned: predominance of eurybiont nonspecific species (*Cryptococcus albidus*, *Debaryomyces hansenii*) in spring, mass development of specific nectar-associated yeasts (*Metschnikowia reukaufii*) in summer, and their substitution by widespread epiphytic species (*Rhodotorula mucilaginosa*, *Cryptococcus magnus*) in autumn.
- 5 Kachalkin AV 2014 Isolation of a *Candida saitoana* divergent strain from the Anyui mummy of a steppe bison (*Bison priscus*). *Microbiology* 83:296–298.
In 2012, in the course of the investigation of the yeasts associated with the Pleistocene bison (steppe bison) mummy found recently in permafrost in the lower reach of Anyui River (Chukotka, Russia), the strain *Candida saitoana* VKPM Y-3988 was isolated, which had substantially divergent rDNA nucleotide sequences, as well as certain specific phenotypic features.
- 6 Abdullabekova DA, Magomedova ES, Kachalkin AV, Magomedov GG, Chernov IYu 2014 Yeasts community structure of vineyard in Dagestan. *Mikologiya i fitopatologiya (Mycology and Phytopathology)* 48:80–85 (in Russian).
Yeasts communities inhabiting the vineyard in the Republic Dagestan were studied. The research showed that the different types of substrates associated with vineyard differed by the seasonal dynamics of yeasts numbers. The maximum numbers of yeasts were observed on the leaves and in the litterfall at the end of vegetation. We isolated 20 species of yeasts from the different types of substrates associated with vineyard, 17 of them represented by ascomycetous yeasts. The maximum species richness values among of analysed samples were observed on berries. Our research showed that the type of the seasonal dynamics of yeasts communities formed on fleshy sugar-containing fruits are quite similar regardless of the different regions, i. e. epiphytic yeasts dominate on the young fruits, then the relative abundance of yeasts of the genera *Hanseniaspora* and *Metschnikowia* increase as the fruits ripened. The results demonstrated that yeasts communities formed on the grapes include species which are capable of active fermentation processes.

- 7 Kachalkin AV 2014 Yeasts of the White Sea intertidal zone and description of *Glaciozyma litorale* sp. nov. Antonie van Leeuwenhoek (in press).

The intertidal yeast communities inhabiting various environments in the territories of the White Sea Biological Station “Kartesh” (WSBS ZIN RAS) and the N.A. Pertsov White Sea Biological Station (WSBS MSU) were studied. A total of 31 yeast species were isolated using a conventional plating technique and identified using molecular methods. The yeast community of the White Sea intertidal zone consists of members that are typical for marine substrates, ubiquitous species that are common in water and in low-temperature terrestrial environments, and a group of species that was isolated from marine

substrates for the first time. The most diverse yeast communities formed on the surface of marine algae and in silt. *Metschnikowia zobellii*, which is a typical inhabitant of northern seas, was the most abundant yeast on algae from both biological stations. A new basidiomycetous yeast species, which was described in this work as *Glaciozyma litorale* sp. nov., dominated in the silt samples. The type strain of this new species is K94bT (=KBP 4246T = VKPM Y-3850T = PYCC 6252T = CBS 12957T = DSM 28204T); MycoBank registration number is MB 805475.

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Recent publications.

- 1 Della-Bianca BE, Gombert AK 2013 Stress tolerance and growth physiology of yeast strains from the Brazilian fuel ethanol industry. Antonie Van Leeuwenhoek 104:1083-95 - doi: 10.1007/s10482-013-0030-2.
- 2 Della-Bianca BE, Basso TO, Stambuk BU, Basso LC, Gombert AK 2013 What do we know about the yeast strains from the Brazilian fuel ethanol industry? Appl Microbiol Biotechnol 97:979-91 - doi: 10.1007/s00253-012-4631-x.

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The following review papers have been published recently.

- 1 Johnson EA 2013 Biotechnology of non-*Saccharomyces* yeasts—the Ascomycetes. Appl Microbiol Biotechnol 97:503–517 - DOI 10.1007/s00253-012-4497-y

Saccharomyces cerevisiae and several other yeast species are among the most important groups of biotechnological organisms. *S. cerevisiae* and closely related ascomycetous yeasts are the major producer of biotechnology products worldwide, exceeding other groups of industrial microorganisms in productivity and economic revenues. Traditional industrial attributes of the *S. cerevisiae* group include their primary roles in food fermentations such as beers, cider, wines, sake, distilled spirits, bakery products, cheese, sausages, and other fermented foods. Other long-standing industrial processes involving *S. cerevisiae* yeasts are production of fuel ethanol, single-cell protein (SCP), feeds and fodder, industrial enzymes, and small molecular weight metabolites. More recently, non-*Saccharomyces* yeasts (non-conventional yeasts) have been utilized as industrial organisms for a variety of biotechnological

roles. Non-*Saccharomyces* yeasts are increasingly being used as hosts for expression of proteins, biocatalysts and multienzyme pathways for the synthesis of fine chemicals and small molecular weight compounds of medicinal and nutritional importance. Non-*Saccharomyces* yeasts also have important roles in agriculture as agents of biocontrol, bioremediation, and as indicators of environmental quality. Several of these products and processes have reached commercial utility, while others are in advanced development. The objective of this mini-review is to describe processes currently used by industry and those in developmental stages and close to commercialization primarily from non-*Saccharomyces* yeasts with an emphasis on new opportunities. The utility of *S. cerevisiae* in heterologous production of selected products is also described.

- 2 Johnson EA 2013 Biotechnology of non-*Saccharomyces* yeasts—the basidiomycetes. Appl Microbiol Biotechnol 97:7563–7577 - DOI 10.1007/s00253-013-5046-z

Yeasts are the major producer of biotechnology products worldwide, exceeding production in capacity and economic revenues of other groups of industrial microorganisms. Yeasts have wide-ranging fundamental and industrial importance in scientific, food, medical, and agricultural disciplines (Fig. 1). *Saccharomyces* is the most important genus of yeast from fundamental and applied perspectives and has been expansively studied. Non-*Saccharomyces* yeasts (nonconventional yeasts) including members of the Ascomycetes and Basidiomycetes also have substantial current utility and potential applicability in biotechnology. In an earlier minireview, "Biotechnology of non-*Saccharomyces* yeasts—the ascomycetes" (Johnson Appl Microb Biotechnol 97: 503– 517, 2013), the extensive biotechnological utility and potential of ascomycetous yeasts are described. Ascomycetous yeasts are particularly important in food and ethanol formation, production of single-cell protein, feeds and fodder, heterologous production of proteins and enzymes, and as model and fundamental organisms for the delineation of genes and their function in mammalian and human metabolism and

disease processes. In contrast, the roles of basidiomycetous yeasts in biotechnology have mainly been evaluated only in the past few decades and compared to the ascomycetous yeasts currently have limited industrial utility. From a biotechnology perspective, the basidiomycetous yeasts are known mainly for the production of enzymes used in pharmaceutical and chemical synthesis, for production of certain classes of primary and secondary metabolites such as terpenoids and carotenoids, for aerobic catabolism of complex carbon sources, and for bioremediation of environmental pollutants and xenotoxins. Notwithstanding, the basidiomycetous yeasts appear to have considerable potential in biotechnology owing to their catabolic utilities, formation of enzymes acting on recalcitrant substrates, and through the production of unique primary and secondary metabolites. This and the earlier mini-review (Johnson Appl Microb Biotechnol 97:503–517, 2013) were motivated during the preparation and publication of the landmark three-volume set of "The yeasts: a taxonomic study, 5th edition" (Kurtzman et al. 2011a, b).

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Recent publications in proposal of new yeast species proposed by our group.

- 1 Kaewwichian R & S Limtong 2014 *Nakazawaea siamensis* f.a., sp. nov., a novel yeast species isolated from phylloplane in Thailand. Int J Syst Evol Microbiol 64:266–270 - DOI:10.1099/ijms.0.057521-0
- 2 Junyapate K, S Jindamorakot & S Limtong 2014 *Yamadazyma ubonensis* f.a., sp. nov., a novel xylitol producing yeast species isolated in Thailand. Antonie van Leeuwenhoek 105: 471–480.
- 3 Chamnanpa T, P Limtong, N Srisuk, S Limtong 2013 *Pseudozyma vetiver* sp. nov., a novel anamorphic ustilaginomycetous yeast species isolated from the phylloplane in Thailand. Antonie van Leeuwenhoek 104:637–644.
- 4 Limtong S & R Kaewwichian 2013 *Candida phyllophila* sp. nov. and *Candida vitiphila* sp. nov., two novel yeast species from grape phylloplane in Thailand. J Gen Appl Microbiol 59:191–197.
- 5 Kaewwichian R, H Kawasaki & Limtong S 2013 *Wickerhamomyces siamensis* sp. nov., a novel yeast species isolated from phylloplane in Thailand. Int J Syst Evol Microbiol. 63:
- 6 Kaewwichian R, W Yongmanitchai, H Kawasaki, P-H Wang, S-H Yang & S Limtong 2013 *Yamadazyma siamensis* sp. nov., *Yamadazyma phyllophila* sp. nov. and *Yamadazyma paraphyllophila* sp. nov., three novel yeast species isolated from phylloplane in Thailand and Taiwan. Antonie van Leeuwenhoek 103:777-788.

- 7 Limtong S, R Kaewwichian & Groenewald M 2013 *Ogataea kanchanaburiensis* sp. nov. and *Ogataea wangdongensis* sp. nov., two novel methylotrophic yeast species from phylloplane in Thailand. *Antonie van Leeuwenhoek* 103:551–558.
- 8 Kaewwichian R, W Yongmanitchai, H Kawasaki & S Limtong 2012 *Metschnikowia saccharicola* sp. nov. and *Metschnikowia lopburiensis* sp. nov., two novel yeast species isolated from phylloplane in Thailand. *Antonie van Leeuwenhoek* 102:743–751.
- 9 Limtong, S, S Nitiyon, R Kaewwichian, S Jindamorakot, S Am-In, & W Yongmanitchai 2012 *Wickerhamomyces xylosica* sp. nov. and *Candida phayaonensis* sp. nov., two novel xylose-assimilating yeast species isolated in Thailand. *Int J Syst Evol Microbiol* 62:2786–2792.
- 10 Limtong S, N Koowadjanakul, S Jindamorakot, W Yongmanitchai & T Nakase 2012 *Candida sirachaensis* sp. nov. and *Candida sakaeoensis* sp. nov. two anamorphic yeast species from phylloplane in Thailand. *Antonie van Leeuwenhoek* 102:221–229.
- 11 Limtong S, R Kaewwichian, S Jindamorakot, W Yongmanitchai, & T Nakase 2012 *Candida wangnamkhiaoensis* sp. nov., an anamorphic yeast species in *Hyphopichia* clade isolated in Thailand. *Antonie van Leeuwenhoek* 102:23–28.
- 12 Nakase T, S Jindamorakot, S Am-In, S Ninomiya, H Kawasaki & S Limtong 2011 *Candida maleeae* sp. nov., a novel anamorphic yeast species in the *Ambrosiozyma* clade found in Thailand. *J Gen Appl Microbiol* 57:253–258.
- 13 Koowadjanakul N, S Jindamorakot, W Yongmanitchai & S Limtong 2011 *Ogataea phyllophila* sp. nov., *Candida chumphonensis* sp. nov. and *Candida mattranensis* sp. nov., three methylotrophic yeast species from phylloplane in Thailand. *Antonie van Leeuwenhoek* 100:207–217.
- 14 Limtong S, S Jindamorakot, S Am-In, R Kaewwichian, S Nitiyon, W Yongmanitchai & T Nakase 2011 *Candida uthaithanina* sp. nov., an anamorphic yeast species in *Nakaseomyces* clade isolated in Thailand. *Antonie van Leeuwenhoek* 99:865–871.

Other recent publications.

- 15 Limtong S & R Kaewwichian 2014 The diversity of culturable yeasts in the phylloplane of rice in Thailand. *Annals Microbiol* - doi:10.1007/s13213-014-0905-0.
- 16 Limtong S, R Kaewwichian, W Yongmanitchai & H Kawasaki 2014 Yeasts in phylloplanes of sugarcane in Thailand and their capability to produce indole-3-acetic Acid. *World J Microbiol Biotechnol* - doi:10.1007/s11274-014-1602-7.
- 17 Yuangsaard N, W Yongmanitchai, M Yamada & S Limtong 2013 Selection and characterization of a newly isolated thermotolerant *Pichia kudriavzevii* for ethanol production at high temperature from cassava starch hydrolysate. *Antonie van Leeuwenhoek* 103:577–588.
- 18 Pimpakan P, Yongmanitchai W & S. Limtong 2012 Bioethanol production from sugar cane syrup by thermotolerant yeast, *Kluyveromyces marxianus* DMKU3-1042 using fed-batch and repeated batch fermentation in non-sterile system. *Kasetsart J (Natural Science)* 46:1–10.
- 19 Limtong S & N Koowadjanakul 2012 Yeasts from phylloplanes and their capability to produce indole-3-acetic acid. *World J Microbiol Biotechnol* 28:3323–3335.
- 20 Kraisintu P, W Yongmanitchai & S Limtong 2010 Selection and optimization for lipid production of newly isolated oleaginous yeast, *Rhodospiridium toruloides* DMKU3-TK16. *Kasetsart J (Natural Science)* 44:436–445.

Recent relevant publications:

- 1 Stewart GG 2010 The ASBC Award of Distinction Lecture - High gravity brewing and distilling – Past experiences and future prospects. *J Amer Soc Brew Chem* 68:1-9.
 - 2 Stewart GG 2010 Glucose, maltose and maltotriose. Do brewer's yeast strains care which one? In: Proc. 31st Convention of the Institute of Brewing and Distilling, Asia Pacific Section, Paper 03.
 - 3 Stewart GG 2010 MBAA Award of Merit Lecture. A love affair with yeast. *Master Brewers Association of the Americas, Technical Quarterly* 47:4-11.
 - 4 Stewart GG, Andrews JMH, Miedl M and Taylor RJ 2011 The nature and fermentability of last runnings in high gravity brewing. *Master Brewers Association of the Americas, Technical Quarterly* 48:9-12.
 - 5 Bamforth CW and Stewart GG 2011 Brewing - Its evolution from a craft into a technology. *Biologist* 57:139–147.
 - 6 Chlup PH and Stewart GG 2011 Centrifuges in brewing. *Master Brewers Association of the Americas, Technical Quarterly* 48:46-50.
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 - 9 Stewart GG 2012 Biochemistry of brewing. In: *Biochemistry of Foods*. Eskin NAM and Shahidi N (eds). Elsevier, New York, pp. 291-318.
 - 10 Stewart GG and Murray JP 2012 Brewing intensification – successes and failures. *Master Brewers Association of the Americas, Technical Quarterly* 49:111-120.
 - 11 Stewart GG 2013 Yeast management – culture handling between fermentations. In: Proc. 14th Convention of the Institute of Brewing and Distilling, Africa Section. Paper No. 12.
 - 12 Stewart GG, Hill A and Lekkas C 2013 Wort FAN – Its characteristics and importance during fermentation. *J Amer Soc for Brew Chem* 71:179-185.
 - 13 Stewart GG, Hill AE and Russell I 2013 125th Anniversary of the IBD Review - Developments in brewing and distilling yeast strains. *J Inst Brewing* 119:202-220.
 - 14 Stewart GG 2014. Yeast mitochondria – Their influence on brewer's yeast fermentation and medical research. *Master Brewers Association of the Americas, Technical Quarterly* 51:3-11.
 - 15 Lekkas C, Hill AE and Stewart GG 2014 Extraction of FAN from malting barley during malting and mashing. *J Amer Soc Brew Chem* 72:6-11.
 - 16 Stewart GG 2014. *Saccharomyces*. In: *Encyclopedia of Food Microbiology*, 2nd Edition, Catt C and Tortorello ML (eds.), Elsevier, Oxford, UK. Vol 3, pp 297-315.
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Recently accepted papers.

- 1 Lachance MA, Fedor AN 2014 Catching speciation in the act: *Metschnikowia bowlesiae* sp. nov., a yeast species found in nitidulid beetles of Hawaii and Belize. *Antonie van Leeuwenhoek* 105:541-550.

We describe the species *Metschnikowia bowlesiae* sp. nov. based on the recovery of six isolates from Hawaii and Belize. The species belongs to the *Metschnikowia arizonensis* subclade of the large-spored *Metschnikowia* clade. The isolates are haploid and heterothallic. Both Hawaiian strains had the mating type h+ and the Belizean strains were h-. Paraphyletic species structures observed in some ribosomal DNA sequence analyses suggest that

M. bowlesiae sp. nov. might represent an intermediate stage in a succession of peripatric speciation events from *Metschnikowia dekortorum* to *Metschnikowia similis* and might even hybridize with these species. The type of *M. bowlesiae* sp. nov. is strain UWOPS 04-243x5 (CBS 12940T, NRRL Y-63671) and the allotype is strain UWOPS 12-619.1 (CBS 12939A, NRRL Y-63670).

- 2 Daniel HM, Lachance MA, Kurtzman CP 2014 On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription. *Antonie van Leeuwenhoek* - DOI 10.1007/s10482-014-0170-z

- 3 Sousa FMP, Morais PB, Lachance MA, Rosa CA 2014 *Hagleromyces* gen. nov., a yeast genus in the Saccharomycetaceae, and description of *Hagleromyces aurorensis* sp. nov. isolated from water tanks of bromeliads in Brazil (accepted May 2014).

Three strains of a novel yeast species were isolated from water tanks (phytotelmata) of a bromeliad species collected in the state of Tocantins, Brazil. Analysis of sequences for the region spanning the small subunit (SSU) rRNA gene, the internal transcribed spacers (ITS), the 5.8S rRNA gene and the D1/D2 domains of the large subunit rRNA gene and RNA polymerase II gene showed that these novel yeasts belong to a species that is distinct from all currently recognized ascomycetous yeast species.

Based on the results of sequence analyses we propose a novel species representing a new genus in the Saccharomycetaceae. The novel species is assigned to the genus *Hagleromyces* gen. nov. The three isolates of the novel yeast species failed to form sexual spores alone or in mixtures. The name *Hagleromyces aurorensis* sp. nov. is proposed to accommodate the species. The type strain of *H. aurorensis* sp. nov. is UFMG-CM-Y311 (= CBS 13264).

Reflexions on Life and Death

Jure Piškur 1960-2014



This letter, written in mid-May 2014, can be distributed to my family members, friends, collaborators, or anyone who might be interested, etc. It can also be read during my “in memoriam” events and ISSY 31 etc.

We are born in this world for a certain period, which we then fill with joy, work, ambitions, sorrow, love, hate, etc., or just nothing, share these with other people, but then comes time when we have to resign. This is the law of Nature. Sooner or later we are sucked back into this big Recycling Machine, and become parts and energy of new molecular complexes and even new individuals. This is how I, as a life scientist, and not being religious, have understood my existence. And I am grateful that my life period has been long enough that I could experience many strong emotions, like being loved and could love, enjoy my existence and interact with other people, be a part of an intensive family life, admire and enjoy nature, and fulfill my scientific curiousness.

However, it has always bothered me when is the “right time” to depart.....

I grew up in former Yugoslavia, in a small family which lost his other boy Damien when he was only 3 years old. My grand-mother Rozalija was recycled, much before I was born, as a 50 years old lady at one of the concentration camps. My father, a freedom fighter, could hardly survive the war and had on several occasions during his battles escaped death. Thus the smell of early departures of our family members was hanging around. This perhaps urged me to live “fast”, and to fill every day with so many things as possible not to miss anythingbecause the departure day felt to be so unpredictable. I also experienced a totalitarian state in ex-Yugoslavia with its suppression mechanisms. And even this state, luckily, had to depart before it turned fifty.

I still consider my childhood in my home country as something most beautiful I have been given. And Slovenia has always brought me new inspirations, challenges and joy. When one stands in front of the St. Jurij church in Piran, and the tower and its angel touch the blue sky, and your eyes rest over the Adriatic sea.....how much closer to Heaven can one come? This has been one of my favorite spots. Full of spirituality where different energies mix and where one can in his/her mind surf in the time, forwards and backwards, and “meet” almost anyone one desiresgrand grand-fathers and grand grand-children....a time-less cocktail of people.

When still in Slovenia I started my life science explorations, and turned into a biologist and chemist. This was a great decision, an eternal source for my later creativity. I could during my life-span experience really big events in biological sciences, just to mention the start of DNA recombinant techniques and the genomic era. I also met at the University Judita and shared my life with her for over 30 years. Also my first son is from this period. How happy we were even as a poor young family.

And I am still very proud of my active contribution towards the independence of Slovenia. What a privilege it is for an individual to actively experience, after one thousand years of “slavery”, that your country becomes free, respected, and can finally determine its fate.

As a student I visited Scandinavia a few times, but my really large sailing journey started in early 1986. I had the privilege to study and do research in some of the finest academic institutions. I turned from a Ljubljana/Stockholm chemistry-biology hybrid into a molecular geneticist during my Ph.D. period in the Australian National University. Then I did my postdoc at Carlsberg. This institution, I was from then on always been most tightly attached to, fully converted me into a yeast researcher. I stayed shortly at

Stanford, and later, only 31 years old, started establishing my own laboratory groups, first at the University of Copenhagen, then Technical University of Denmark, Lund, University of Nova Gorica and just recently I should return to Copenhagen and start there again. How full of energy I was only a year ago, a high flying little bird. However, Nature can come to you with the bill at any time. A ticket for the Recycling machine can be issued at any time..... Thus, live fast and productive lifeno rest.....

Whether, is 53 enough or not, there has been a lot of joy to educate young people, meeting other bright researchers, continuously trying to find my own path in biological sciences, perform experiments, analyze the results, create new hypotheses, getting recognition from the community. It has been a great life I have had, never being too tired to go to the lab, never too sleepy not to dream about my molecules.

Apart from the sciences there have also been many friends from all around the world with whom I have had the warmest relationships. I have had the privilege to be liked and respected by many. I have been privileged to put my best into many people and also received so much input, on all levels, from other people. And I have had a great supporting family standing behind my achievements, understanding me and pushing forward.

In late 2013 my life started changing. Suddenly I could not control my body anymore, my nights became sleep-less, the first strong pain appeared. I felt so tired, just like suddenly my energy started disappearing. Just like a car running out of fuel. In January I could not run so much anymore, and I lost control over my abdominal part. Day after day I was weaker, and my head became filled with black thoughts. Just like the end of the World is approaching.....something calling me away. The control was lost but could it be regained? In late February I got my diagnosis, cancer, and they discovered a large primary tumor. A few weeks after they found metastases. Each April day became filled with more pain and bad prognosis. Secondary effectsMy life quality became really low, and I started feeling like I am a rotten piece of meat. I lost over 10 kg and now can hardly walk 100m. Many of my last days I just lay in bed, and I am completely dependent on others. However, I have two big helpers and supporters, Judita and Jure Jr. who still try to make my days bearable, help me endlessly and still respect and love me. Also Jan is warmer with me. However, what a fast decay it has been. Just like a bird shot in the sky and following gravitation laws only.

My spring activities and last traces of energy have now been to secure my lab members a decent future progress. I have tried hard to secure the family future economy. And I have tried to live with my growing disabilities, lack of energy, growing disrespect for my body, inability to improve the things. Apart from the family, many people have tried constantly to cheer me up, to persuade me that there may be a light at the end of my black tunnel. Many thanks to Birgitte, Concetta, Dino, Lidja, Lili, Marjeta, Romana, Sofia, Tinkara, etc., for your support and interest in my well being to the bitter end.

However, it has always bothered me when is the right time to depart. When the time is really out and the life quality below any human dignity, when you can really not give anything to the people around you and to yourself, and each new day is just a burden and aim-less, and when does it become more meaningful to recycle yourself into new molecular and energy complexes, and also your closest people can start a new period, a period which is post-you....

Love you,

j

Recent meeting

41th Annual Conference on Yeasts of the Czech and Slovak Commission on Yeasts, Smolenice, Slovakia, May 20-23, 2014

The 41th Annual Conference on Yeasts, organized by the Czech and Slovak Commission on Yeasts, Institute of Chemistry, Slovak Academy of Sciences and Department of Biochemical Technology, Slovak University of Technology, took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, May 20-23, 2014. Prof. M. Sipiczki from the University of Debrecen, Hungary, was invited to present the opening memorial lecture in honour of Dr. A. Kocková-Kratochvílová. Before the lecture he

was awarded a Honorary Membership of the Czechoslovak Society for Microbiology. The conference was attended by 78 scientists, mainly from Czech Republic and Slovakia, however, there were also participants from Austria, Canada, France, Germany, Hungary, Poland, Serbia, Spain and UK, among them several invited speakers.

The program consisted of three sessions dedicated to Biotechnology, Molecular and Cell Biology and Medical Mycology. 25 oral presentations were complemented with 35 posters. 10 posters were selected for 5 min oral presentations as “poster highlights” Interesting scientific program also contained wine presentation and wine tasting by two Slovak wine-producing companies, Včelco Ltd. from Smolenice and Víno Kmeťo, a small private company from the Low Carpathian region.

On a meeting of the Committee of the Czech and Slovak Yeast Commission, which took place during the Conference it was decided that the 42nd Annual Conference on Yeast will be organized in the Smolenice Castle on May 19-22, 2010. Its program will cover Genetics and Molecular Biology of Yeasts, Cell Biology, Medical Mycology and Biotechnology. Further information about the activities of the Czech and Slovak Commission for Yeasts can be found on the website www.chem.sk/yeast. The titles of the lectures and posters presented at the 41th Annual Conference on Yeast are listed below:

Invited Lecture to the memory of Dr. A. Kocková-Kratochvílová

- M. Sipiczki (Hungary): Diverse pools of rRNA genes in *Metschnikowia* species: diversity in regions that determine hairpin-loop structures and evolution by reticulation.

Lectures in the session Yeast Biotechnology

- RJ Weselake, G Chen, X Pan, RMP Siloto, K Caldo, Q Liu, MS Greer, YXu (Canada): Triacylglycerol-biosynthetic enzymes in yeast and plants, and strategies for boosting biomass oil content.
- B Koch, C Schmidt, B Ploier, G Daum (Austria): To be or not to be: minimal changes in the C-terminus affect functionality and stability of yeast triacylglycerol lipase Tgl3p.
- R Holič, P Seč (Slovakia): Lipid homeostasis and fatty acid secretion in the yeast *Saccharomyces cerevisiae*.
- I Malcová-Janatová, L Senohrádková, T Groušl, H Hašek (Czech Republic): Heat shock-induced protein accumulations in *Saccharomyces cerevisiae* and their analysis using a new software application oCellaris.
- Z Kosour, P Jagoš, L Málek, I Malec, H Sychrová, O Zimmermannová, J Zahrádka, T Groušl, J Hašek, I Malcová (Czech Republic): oCellaris – a new tool for automated analysis of microscopy images of yeast cells.
- T Rossignol, C Leplat, JM Nicaud (France): Engineering *Yarrowia lipolytica* for lipid production.
- P Gajdoš, J-M Nicaud, M Čertík (Slovakia/France): Construction of recombinant *Yarrowia lipolytica* for conversion of industrial residues into value added products.

- P Biely, M Vršanská (Slovakia): Diverse biotechnological potential of *Aureobasium pullulans*.
- S Petrik, S Obruca, P Benesova, I Márová (Serbia/Czech Republic): Bioconversion of pretreated spent coffee grounds by selected carotenogenic yeasts.
- I Kolouchova, O Schreiberova, K Sigler, J Masak (Czech Republic): The influence of resveratrol and its structural analogues on biofilm formation and stability of yeast strains.
- O Schreiberová (Czech Republic): The influence of biologically active natural substances on the adhesive properties and EPS composition of *Candida parapsilosis*.

Lectures in the session Molecular and Cell Biology

- K Maeda, K Anand, A Chiapparino, M Kaksonen, A-C Gavin (Germany): Interactome map uncovers phosphatidylserine transport by oxysterol-binding proteins.
- Z Šimová, R Holič, S Cockroft, V Pevala, K Poloncová, D Tahotná, P Griač (Slovakia/UK) Phosphatidylinositol binding of the yeast Pdr16p is essential in response to azole treatment.
- E Goffa, Z Šimová, I Jančíková, P Griač, Y Gbelská (Slovakia/Czech Republic) The impact of KIPdr16p on the *Kluyveromyces lactis* plasma membrane properties.
- J Ariño, A Serra, S Petrezsélyová, I Guevara, Ramos J (Spain): Co-regulation of PHO89 and ENA1 under high pH stress in *S. cerevisiae*.
- J Zemančíková, H Sychrová (Czech Republic) Characterization and comparison of physiological properties and osmotolerance of two *Dekkera bruxellensis* strains.

- ABurger-Kentischer, D Finkelmeier, J Bauer, H Eickhoff, G Kleymann, A Rayyan, K Schröppel, KH Wiesmüller, S Rupp (Germany): A screening assay based on host-pathogen interaction models identifies a set of novel antifungal benzimidazole derivatives and their target.
- K Vaskovicova, V Stradalova, A Efenberk, M Opekarova, J Malinsky (Czech Republic) Assembly of fission yeast eisosomes at the plasma membrane of budding yeast.
- V Pevala, DFričová, J Bellová, N Kunová, J Košťan, L Krejčí, L Tomáška, J Nosek, E Kutejová (Slovakia/Austria/ Czech Republic): The potential role of Mgm101 protein from *Candida parapsilosis* in the maintenance of mitochondrial telomeres.

Lectures in the session Medical Mycology

- K Kuchler (Austria): Molecular basis of fungal pathogenesis – Staying cool is key to survival.
- A Munro, LA Walker, KK Lee, S Alawfi, K Nather, DM MacCallum (UK): Fungal cell wall remodelling and antifungal drug tolerance.
- J Turánek, PKnotigová, H Čelechovská, E Bartheldyová, J Mašek, P Kulich, R Lukáč, I Lipenská, D Mašková, E Paulovičová, M Ledvina, A D Miller, M Raška (Czech Republic/Slovakia): Mannan – molecular adjuvans for liposome recombinant vaccines.
- E Bartheldyová, JMašek, P Kulich, P Knotigová, HČelechovská, R Lukáč, I Lipenská, D Mašková, EPaulovičová, M Ledvina, M Raška, AD Miller, JTuránek(Czech Republic/Slovakia/UK): Liposomal mannan – preparation and characterisation by electron microscopy and physical-chemical methods.
- A Kasperova, R Cahlikova, J Kunert, M Sebela, J Masek, E Bartheldyova, M Krupka, J Turanek, M Raška (Czech Republic): The regulation of cysteine dioxygenase activity in dermatophyte *Trichophyton mentagrophytes*.
- H Čuláková, V Džugasová, R Valenčíková, YGbelská, J Šubík (Slovakia) The expression of selected multidrug resistance genes in *Candida glabrata* cells deleted in CgPDR16.
- D Kregiel, J Berłowska, H Antolak (Poland): Simplified evaluation of yeast autolysis using the Muse® cell analyzer.
- I Vopálenská, B Janderová, L, Váchová, Z Palková (Czech Republik): A new sensor system for detection of heavy metal ions.
- E Horváth, WP Pfliegler, M Sipiczki (Hungary): Taxonomic analysis of antagonistic *Metschnikowia* strains suitable for bioprotection.
- M Vinarčíková, P Jaká, M Mentel, P Polčic (Slovakia) Characterization of activity of Ybh3 protein in yeast *Saccharomyces cerevisiae*.
- L Hatakova, L Vachova, Z Palkova (Czech Republic) Gcn4p regulation of amino acid metabolism during development of yeast colonies.
- K Kováčová, V Farkaš, L Popolo (Slovakia/Italy)The biochemical characterization of Phr1 and Phr2 transglycosylases of the *Candida albicans* cell wall.
- K Papoušková, M Andršová, H Sychrová (Czech Republic) The putative ion channel Ist2 is important for the maintenance of cell alkali-metal-cation homeostasis.
- H Antolak, D Kregiel (Poland): Anti-adhesive properties of crunberry juice.
- A Kunicka-Styczyńska, M Maroszyńska, K Rajkowska (Poland): Adhesion abilities of *Candida* strains in essential oils presence.
- M Vršanská, S Voběrková, R Vadkertiová, E Stratilová, J Omelková (Czech Republic/ Slovakia): The characterization of lipases produced by yeasts.
- I Bénes, K Furdíková, D Šmogrovičová, J Lakatošová (Slovakia): Influence of yeast strain on sensory profile of acacia and blossom mead.
- I Márová, A Hároniková, N Mikheichyk, S Petrik, V Hlaváček (Czech Republik): Production of some lipid-soluble metabolites by red yeasts cultivated on rapeseed cake.
- I Kostovová, A Hároniková, S Petrik, I Márová Ivana (Czech Republik): Optimisation of DGGE nested PCR for red yeasts characterization.
- K Makyšová, K Furdíková, I Špánik (Slovakia): Influence of autochthonous yeast strains on sensory profile of Gewürztraminer wine.
- M Zichová, L Babák, M Rosenberg, E Stratilová, J Omelková (Czech Republik): Ethanol production from waste paper using immobilized *Saccharomyces cerevisiae* cells.
- R Vadkertiová, J Molnárová1, A Lux, D Lišková, M Vaculík (Slovakia): The tolerance of yeasts to various chemical elements.

Posters

- V Hlaváček, A Hároniková, M Pala, N Mikheichyk, S Petrik, I Márová (Czech Republik/Serbia): Biotechnological conversion of rapeseed cake residue.
- D Gogová, P Gajdoš, M Čertík, J-M Nicaud (Slovakia/France): Biodiesel production by recombinant *Yarrowia lipolytica* cultivated on glycerol.

- V Plocek, L Váchová, Z Palková (Czech Republik): Regulation of Ato protein production during development of yeast colonies.
- K Rajkowska, A Kunicka-Styczyńska, M Maroszyńska (Poland): Cell surface hydrophobicity of *Candida* clinical strains.
- B Hušeková, H Elicharová, H Sychrová (Czech Republik): transport systems for potassium uptake in *Candida albicans*.
- M Bucova, M Suchankova, E Paulovicova, L Paulovicova, E Tibenska, I Majer, Tedlova EH Novosadova (Slovakia): Antifungal antibodies in bronchoalveolar lavage fluid in patients with pulmonary sarcoidosis.
- E Paulovičová, L Paulovičová, R Pilišiová, S Bystrický, DV Yashunsky, AA Karelin, YE Tsvetkov, NE Nifantiev (Slovakia/Russia): *Candida albicans* cell wall glycan antigens - novel tools to study host-pathogen interactions.
- E Drozdíková, M Garaiova, M Obernauerová, I Hapala (Slovakia): Influence of squalene epoxidase manipulation on neutral lipid homeostasis in the *Kluyveromyces lactis* yeast.
- J Jäger, B Stratilová, J Molnárová, P Řehulka, J Omelková, R Vadkertiová, EStratilová (Czech Republik/Slovakia): MS based identification of *Cryptococcus laurentii* group.
- M Garaiova, R Holic, M Valachovic, I Hapala (Slovakia): High accumulation of squalene is toxic to yeast cells lacking lipid droplets.
- E Dobáková, J Laco, G Gavurníková, M Mentel, P Polčic (Slovakia): Genetic and biochemical characterization of putative Sall protein of yeast *Schizosaccharomyces pombe*.
- M Balážová, P Griač (Slovakia): The specific amount of phosphatidylglycerol is important for mitochondrial functions in the yeast *Saccharomyces cerevisiae*.
- M Papay, MValachovic, (Slovakia): Anaerobic expression of genes encoding sterol importers.
- N Kunov, J Bellová, V Pevala, Ľ Ambro, E Kutejová (Slovakia/Czech Republik): Mitochondrial nucleoid proteins as novel substrates for Lon-mediated proteolysis in *Saccharomyces cerevisiae*.
- V Llopis-Torregrosa, H Sychrová (Czech Republik): Effect of the alkali metal transporters Ena1, Cnh1 and Trk1 in intracellular pH of *Candida glabrata*.
- V Raclavský, R Novotný, V Kolek (Czech Republik): *Pseudomonas aeruginosa* as a potential source of agents interfering with capsule formation in *Cryptococcus neoformans*.
- WP Pfliegler, E Karanyicz, M Sipiczki (Hungary): Genetic characterization of interspecies *Saccharomyces* di- and trihybrids with emphasis on the fate of mitochondria.
- Z Balážfyová, N Tóth Hervay, A Svrbická, R Káčeriková, Y Gbelská (Slovakia): Yap1p and Pdr1p in the *Kluyveromyces lactis* multidrug resistance modulation.

Communicated by Peter Biely

Forthcoming Meetings

XIVth International Congress of Mycology & Eukaryotic Microbiology Montréal, Québec, Canada, July 27th - August 1st 2014

The Congresses of the International Union of Microbiological Societies (IUMS 2014) will take place from July 27th to August 1st, 2014 at the Palais des Congrès de Montréal (Montréal's Convention Centre), in Montréal, Canada.

The three congresses [XIVth International Congress of Mycology; XIVth International Congress of Bacteriology and Applied Microbiology; XVIth International Congress of Virology] will be held

simultaneously within one week to stimulate cross talk.

The Mycology Division of IUMS is in charge of the International Congress of Mycology and we foresee to expand its scope beyond the fungi. Thus the congress will cover Mycology and other Eukaryotic microorganisms.

For further information, see:

<http://www.montrealiums2014.org/>

Teun Boekhout (Chair of the Mycology congress, vice-chair Mycology Division)

Pierre Belhumeur (Vice-chair of the Mycology congress)

Scott Baker (Former chair Mycology congress 2011, Chair Mycology Division)

10th International Mycological Congress, August 3-8, 2014, Bangkok, Thailand

The 10th International Mycological Congress will be held in Bangkok, Thailand from August 3 to 8, 2014. Although a predominance of hyphal-growth enthusiasts may be counted on, many facets of yeast research will integrate well into the program, for example stress metabolism, insect-fungal symbioses, endophytes, biocontrol, extreme environments, diversity assessments, traditional fermented Asian foods, metabolites, biotechnology and genomes. A session dedicated to “Diversity and molecular

taxonomy of yeasts” will be organised and abstract submission is open till March 31, 2014. It is hoped that a Special Interest Group (SIG) on yeast nomenclatural issues and the future of “The Yeasts: A taxonomic study” will be met with interest and participation by the yeast community. For further information on program and deadlines please refer to www.imc10.com.

We look forward seeing you in Bangkok.

Heide-Marie Daniel, Masako Takashima, and Teun Boekhout

ISSY 31 Yeast Fermentation: From Genes to Application Aspects Vipava, Slovenia 9-12th October 2014

The conference is organized in the renovated Lanthieri Palace by Lund University, University of Nova Gorica, EU FP7 Cornucopia and Jubilekinase

ApS. ISSY 31 is organized under auspices of International Commission on Yeasts (ICY). For further information, consult:

http://www.yeast-cornucopia.se/index.php?option=com_content&view=article&id=12&Itemid=24

Brief News Item

New coordinates: Andreas K. Gombert

Effective March 2013, I have moved from the Bioprocess Engineering Group (GEnBio), Department of Chemical Engineering, University of Sao Paulo to the following:

Bioprocess and Metabolic Engineering Laboratory (LEMeB)
Faculty of Food Engineering (FEA)
University of Campinas (UNICAMP)
Rua Monteiro Lobato, 80
13083-862 Campinas - SP
Brazil

[<gombert@unicamp.br>](mailto:gombert@unicamp.br)

50 Years Ago

Fifty Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

May 1964

Volume XIII, Number 1

Editor

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[Yeast Newsletter Vol XIII, No. 1, May 1964](#)

Miss W. Ch. Sloof of CBS announced that type strains of new yeast species were received at CBS including *Candida bogoriensis*, *C. diffluens*, *C. foliarum*, *C. silvae*, *Chlamydozyma pulcherrima*, *C. reukaufii*, *Endomycopsis fasciculata*, *Fabospora phaffii*, *Pichia saccharophila*, *Saccharomyces nilssoni*, *S. norbensis*, *S. osmophilus*, *Schwanniomyces hominis*, *Torulopsis salmanticensis*, *Trichosporon loboii*.

Mrs. N. J. W. Kreger-van Rij resigned from CBS because her husband moved to the University of Groningen.

Dr. L. J. Wickerham of the US Department of Agriculture, Northern Utilization Research and Development Division communicated the publication of description of a new family of yeasts called Chlamydozymaceae, with type genus *Chlamydozyma*, which exhibit protosexuality, or ability to form a dikaryon or diploid state and return to the haploid state without the formation of ascospores. He also published description of a cadaver-associated yeast, *Hansenula petersonii*, that can tolerate embalming fluid and phenol solutions.

Dr. O. Verona of the Istituto di Patologia Vegetale e Microbiologia Agraria, Pisa, Italy described isolation of yeasts from fishes, other marine animals, wood pulp, and leaf litter, as well as ultrastructure of *Trigonopsis variabilis*.

Prof. J. Boidin of Laboratoire de Microbiologie & Mycologie, Faculté des Sciences, Lyon, France reported publication of the description of *Candida berthetii*, and requested manuscripts, reprints, strains, etc. to aid in their revision of the genus *Pichia*.

Dr. Henri Saëz of the Muséum National d'Histoire Naturelle, Parc Zoologique Paris, France isolated and identified yeasts from feces of young children, and detected *Candida albicans* in healthy individuals. He also reported intestinal mycoflora of 77 young mammals.

J. F. T. Spencer enumerated yeasts in the Saskatchewan River upstream and downstream of Saskatoon, and in a sewage lagoon. Genera included *Rhodotorula*, *Cryptococcus*, *Trichosporon*, *Pichia*, *Candida* and *Saccharomyces*. *S. cerevisiae* was presumably contributed by local breweries, bakeries, and homes where bread was baked. He also reported extracellular glycolipid production by *Rhodotorula*. The doctoral dissertation of P. S. S. Dawson was summarized.

Eng. I. Taysi described a study performed under the guidance of N. van Uden and supported by the Gulbenkian Foundation, Lisbon, Portugal. Yeasts were profiled in rivers Tagus and Sado, and adjacent Atlantic zones.

Dr. H. J. Phaff of the University of California, Davis announced publication of descriptions of tree exudate-associated yeasts *Pichia trehalophila* and *P. salictaria*, and brine-shrimp-associated yeast *Metschnikowia kamienskii*.

Dr. Colin H. Clarke of the Institute of Animal Genetics, Edinburgh, Scotland gave an account of a symposium on yeast mutation and DNA repair at Freiburg im Breisgau, organized by Prof. H. Marquardt and Dr. F. K. Zimmermann.

Dr. Nicola Lopreino of the Istituto di Genetica della Universita, Pisa, Italy described research on mutagenicity of UV and nitroso compounds in *Schizosaccharomyces pombe*.

Dr. Thomas D. Brock of Indiana University studied enzymes that may participate in conjugation in *Hansenula wingei* and other species, particularly in cell wall hydrolysis.

Dr. J. Kleyn, Sicks' Rainier Brewing Co., Seattle, Washington, USA identified beer spoilage yeasts from non-pasteurized beer as *Saccharomyces diastaticus* or related species, because the cells were oval budding cells and produced superattenuation in finished beer. Conference presentations on yeast dwarf cell formation were listed.

The editor (Dr. H. J. Phaff) announced with deep regret the death of **Professor Raffaella Cifferri**, director of the Istituto de Orto Botanico della Universita of Pavia in February, 1964.

The editor also received a new edition of the Catalogue of Cultures (Aug. 1963) from the National Collection of Yeast Cultures, Surrey, England.

Dr. Carl C. Lindegren accepted an appointment as a consultant for the distillery Planta Piloto de Ron in Puerto Rico, working three months per year on yeast problems in the fermentation. Summaries of three publications by the Carlsberg Laboratorium, Copenhagen, Denmark were listed. They studied effects of X-rays on spore germination in *S. pombe*.

Kyria Boundy-Mills, Phaff Yeast Culture Collection, University of California Davis
